

SCR 0395P



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Cochrane et al.

Serial No.: 07/715,397

Filed: June 14, 1989

For: PULMONARY SURFACTANT
PROTEIN AND RELATED
POLYPEPTIDE

Examiner: Perkins

) Group Art Unit: 189

DECLARATION

Hon. Commissioner
of Patents and Trademarks
Washington, D.C. 20231

Dear Sir:

I, Susan D. Revak, declare that:

1. I am a co-inventor of the invention described in the above-identified patent application.

2. Under my direction and control, various synthetic surfactant peptides were synthesized. These synthetic surfactant peptides are designated below, and are followed by their amino acid residue sequences:

RL2 RLLRLLRLLRLLRLLRLL

RL4 RLLLLRLLLLRLLLLRLLLLR

RL4-CYS RLLCLLRLLLLRLLCLLRLLLLRLLLL

RL8 RLLLLLLLLRLLLLLLLLLRL

(RL4)₄R RLLLLRLLLLRLLLLRLLLLR

(RL4)₅R RLLLLRLLLLRLLLLRLLLLRLLLLR

(RL4)₆R RLLLLRLLLLRLLLLRLLLLRLLLLRLLLLR

(RL4)₇R RLLLLRLLLLRLLLLRLLLLRLLLLRLLLLR

(RL4)₈R RLLLLRLLLLRLLLLRLLLLRLLLLRLLLLR

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The peptide sequences correspond to the formulations disclosed in the specification and claimed herein; see, e.g., the formula set forth on page 27, line 1 through page 28, line 15, which includes Table 3, of the above-identified application and also correspond to the sequences set forth in the pending claims. It should also be noted that the sequences for RL4 and RL8 are given on page 28 (in Table 3) of the specification, and that the preferred sequence for RL4, which is given in Table 3 of the application, is the same as the sequences identified above as RL4 and as $(RL4)_4R$. (The abbreviated term "RL4" is generally used to indicate the sequence RLLLLRLLLLRLLLLRLLLLR, albeit the term $(RL4)_4R$ is perhaps more descriptive.)

3. Surfactant solutions containing one of the above-noted peptides ("peptide-containing" or "synthetic" surfactants) were prepared according to the teachings of the above-identified application for use in the studies described herein. Particular solutions will be described further hereinbelow.

4. Under my direction and control, *in vivo* studies were initiated to confirm the efficacy of the novel peptides (and synthetic surfactants containing those peptides) described in the above-noted application. Surfactants consisting of RL2, RL4, RL4-CYS or RL8 peptides, or "multiples" of RL4 (e.g., $(RL4)_4R$) were typically compared to a phospholipid surfactant not containing peptide or protein ("control surfactant"). *In vivo* assessment of synthetic surfactant activity using the fetal rabbit model described at page 55, line 30 through page 58, line 33 was used to study surfactant activity as disclosed in the specification.

5. Using the plethysmograph, dynamic compliance measurements were made at various times throughout a 60 minute ventilation period. Data analysis resulted in compliance data expressed as ml of air per cm H₂O per gram of body weight X 10⁶ at each time point. Compliance was calculated by the formula shown and discussed on

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page 58, lines 3-33 of the specification. The *in vivo* compliance studies demonstrate that the use of a number of exemplary synthetic surfactants of this invention resulted in enhanced compliance in comparison to phospholipid alone for each of the assayed synthetic surfactants. Thus, the proteins and polypeptides of this invention when admixed with pharmaceutically acceptable phospholipids form synthetic surfactants that have greater surfactant activity than phospholipid alone. Use of the synthetic surfactants is advantageous in producing improved compliance values *in vivo*.

6. The data generated from the above-described studies are illustrated in Tables 1, 2, and 3, and in Figure A attached hereto. The Tables and Figure A illustrate the following.

Tables 1 and 2 - the results of studies conducted on April 11, 1989 and April 18, 1989, respectively, are illustrated. Synthetic surfactants RL2, RL4, and RL8 were studied in *in vivo* dynamic compliance tests.

Table 3 and Figure A - Synthetic surfactants RL4-CYS, (RL4)₄R, (RL4)₅R, (RL4)₆R, (RL4)₇R, and (RL4)₈R were studied in *in vivo* dynamic compliance tests conducted from April 30 - May 1, 1990.

7. For the studies illustrated in Tables 1 and 2, the synthetic surfactant solutions were comprised of DPPC, PG and peptide in the following proportions. (See also page 49, line 16 through page 50, line 21 for other surfactant preparation methods.) DPPC: 15 mg/ml; PG: 5 mg/ml; and peptide: 2 mg/ml. In the Table 2 studies, the administered solutions which also contained palmitic acid were designated with a "+" symbol, while those with a "-" did not include palmitic acid. Administration protocol 4 was used in the studies illustrated in Tables 1 and 2. This protocol is described at page 56, lines 13-18 of the specification.

8. The data generated from the above-described studies are further illustrated in Table 3 and Figure A attached hereto. The Table and Figure illustrate the effect of administration of various

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formulations of RL4-containing surfactant on lung function and demonstrate the results of an effort to determine the optimal length of an RL4-containing peptide. In Table 3, the *in vivo* efficacy of a cysteine-containing synthetic surfactant is also assayed. In Table 3 and Fig. A, the average dynamic compliance value for animals tested using the designated formulation is plotted against time in minutes after surfactant administration. The surfactant was administered as described above, with reference to protocol 4.

9. The studies described herein and depicted in the Tables and Figure attached hereto demonstrate that the synthetic surfactants of the present invention display marked therapeutic utility. For example, the dynamic compliance values observed for all the synthetic surfactants significantly exceed those values typically obtained with phospholipid alone. Moreover, as dynamic compliance tests are clearly indicators of *in vivo* efficacy in mammals, including humans, it is not surprising that the herein-described data correlates well with the primate data discussed in the Declaration of Charles Cochrane, which has also been submitted herewith.

10. Furthermore, the therapeutic efficacy demonstrated via these *in vivo* dynamic compliance studies is consistent with the results noted when these same compositions were tested using the "pulsating bubble" *in vitro* assays described and illustrated on pages 48-54 of the specification (which includes Table 7).

11. From these data I conclude that the "pulsating bubble" *in vitro* assays of surfactant activity described in the specification are predictive of *in vivo* efficacy, particularly in light of the fact that the *in vivo* studies described herein and in the specification demonstrate the therapeutic efficacy of the synthetic surfactants conforming to the formulations disclosed in the above-referenced application. The significant improvement in

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dynamic compliance values in these *in vivo* studies underscores the therapeutic value of the disclosed and claimed synthetic surfactants.

12. The studies described in paragraphs 2-11 were performed in the United States.

13. All statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

4-10-92
Date

Susan D. Revak
Susan D. Revak

ACL\C:\WP\OA\REVAKDEC.395

I hereby certify that this correspondence is being deposited with the United States Postal Service as first class mail in an envelope addressed to Commissioner of Patents and Trademarks Washington, D.C. 20231 on

4-13-92

(Date of Deposit)

April C. Logan, Reg. No. 33,950

Name of applicant, assignee or
Registered Representative

April C. Logan

Signature

4-13-92

Date of Signature



TABLE 1

Dynamic Compliance
in ml air/cm H₂O/g body weight x 10⁶

	Minutes after Surfactant Instillation						Sample Given By Protocol #
	10	20	30	40	50	60	
<u>RL2^a</u> +	54	66	120	127	200	247	4
<u>RL4</u> +	216	140	189	241	289	283	4
	34	46	88	114	134	193	4
<u>RL8</u> -	44	80	117	187	228	228	4
	63	80	102	107	245	154	4
	27	37	55	96	113	148	4

a Solutions referenced with a "+" contain palmitic acid, while those referenced with a "-" do not.

TABLE 2

Dynamic Compliance
in ml air/cm H₂O/g body weight x 10⁶

	Minutes after Surfactant Instillation						Sample Given By Protocol #
	10	20	30	40	50	60	
<u>RL2</u>	69	147	226	245	239	253	4
<u>RL8</u>	72	442	442	489	464	464	4
	79	110	135	126	149	169	4

TABLE 3

Dynamic Compliance
in ml air/cm H₂O/g body weight x 10⁶

	Minutes after Surfactant Instillation						Sample Given By Protocol #
	10	20	30	40	50	60	
<u>RL4-CYS</u>							
	20	31	31	47	47	78	4
	34	40	34	48	54	71	4
	40	40	48	51	66	n/d ^b	4
<u>(RL4)₈R</u>							
	75	109	173	PTX ^c	PTX	PTX	4
	32	51	32	37	37	51 (PTX)	4
	42	50	54	65	69	84	4
	46	50	50	54	54	n/d	4
<u>(RL4)₇R</u>							
	35	39	53	53	74	88	4
	30	42	54	83	95	107	4
	37	44	55	55	52	55	4
<u>(RL4)₆R</u>							
	46	39	74	106	124	135	4
	80	92	112	141	149	143	4
	40	52	60	65	65	60 (PTX)	4
<u>(RL4)₅R</u>							
	15	39	66	123	112	73 (PTX)	4
	35	41	56	56	32	53	4
	39	85	72	92	82	99	4
<u>(RL4)₄R</u>							
	38	91	140	206	251	297	4
	39	41	56	56	32	53	4
	38	44	44	48	52	52	4

b n/d = not determined

c PTX = pneumothorax

**Effect of Synthetic Peptide Surfactant on Lung Function
in Premature Neonatal Monkey**

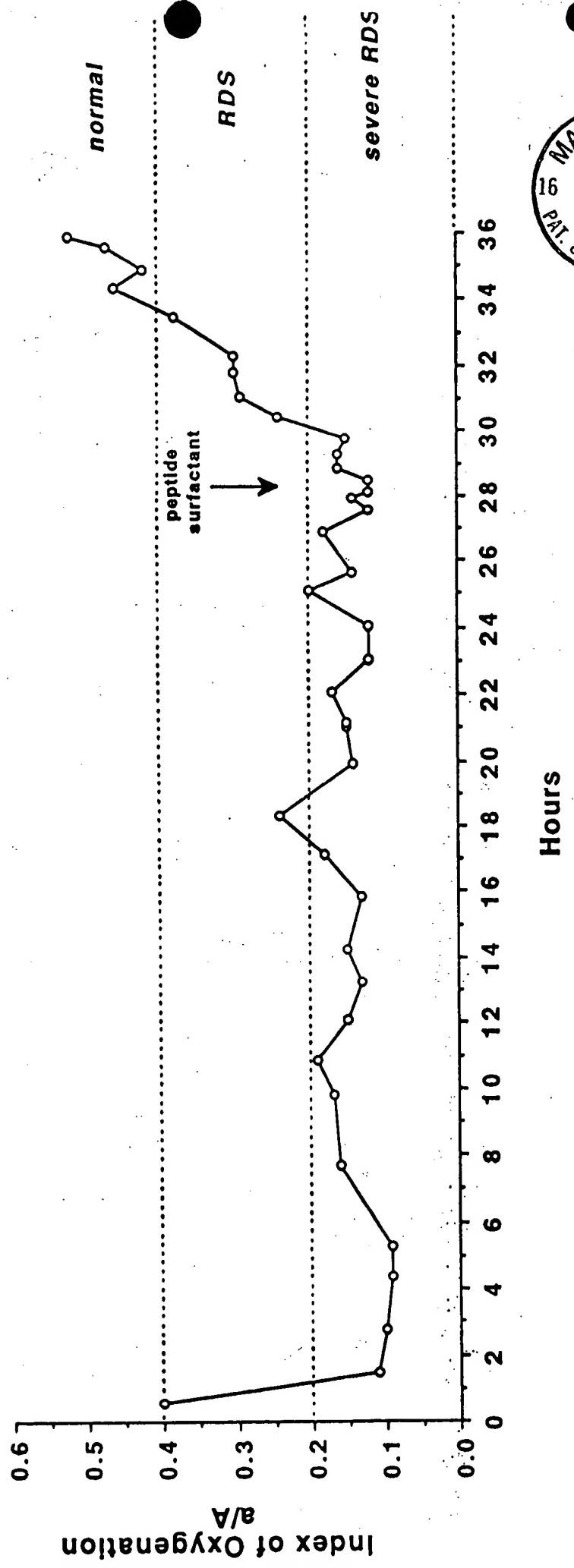


Fig. 1

OPTIMAL LENGTH OF Best Available Copy - FETAL RABBIT

461510

(n = 3 or 4)

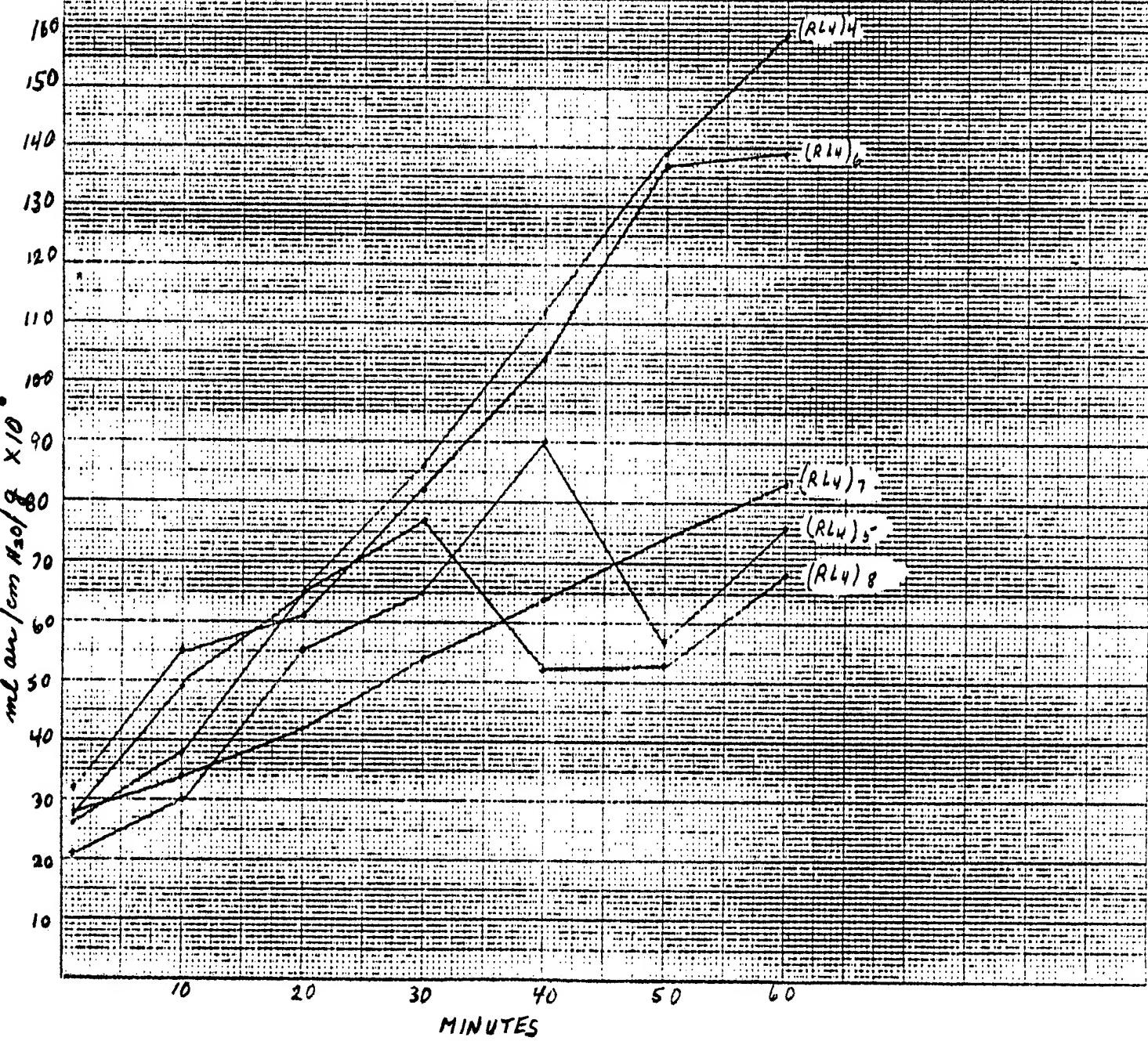


Fig. A

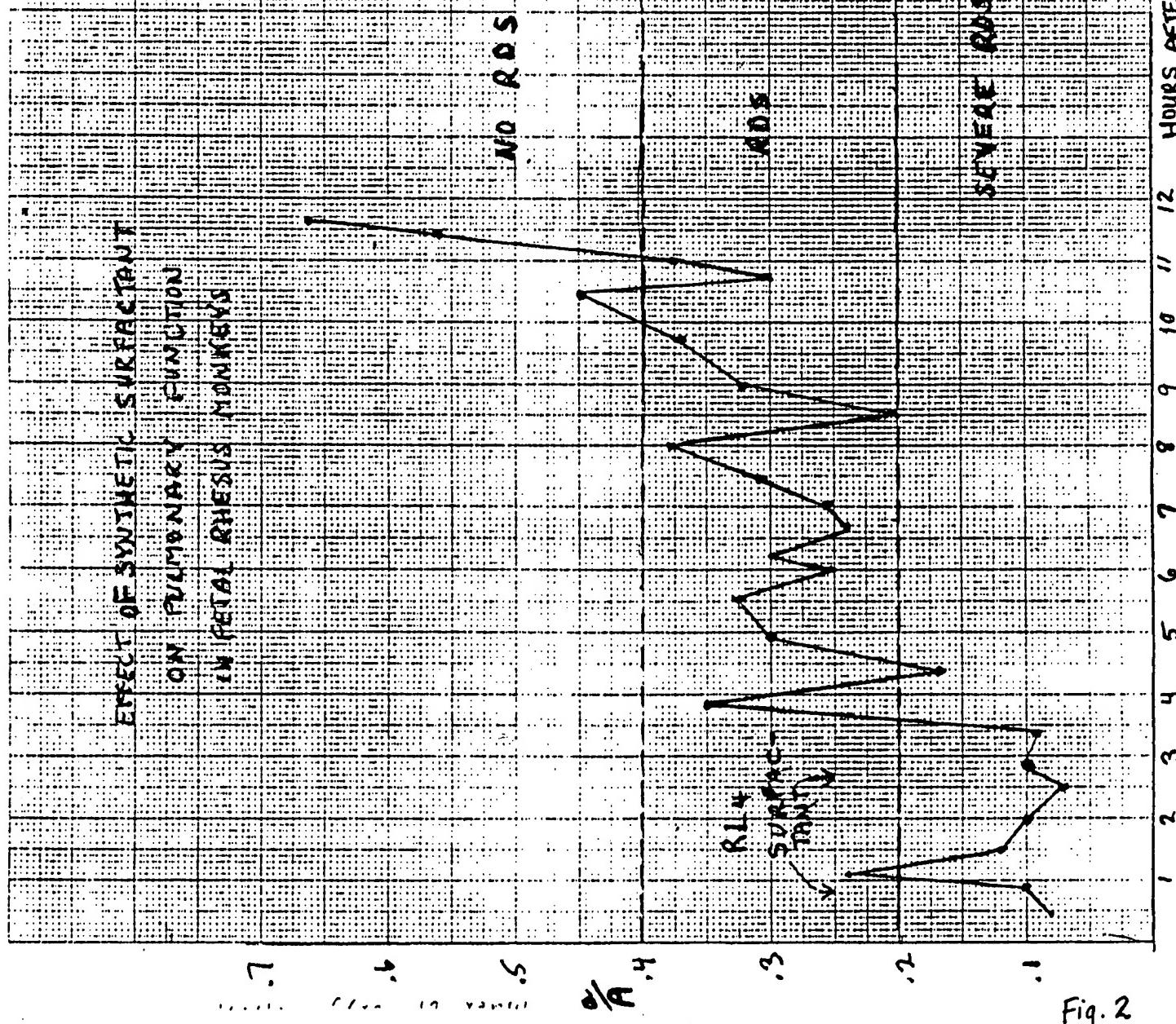
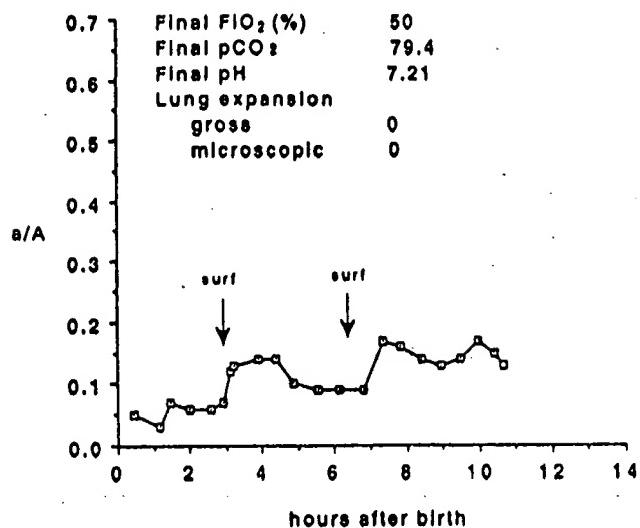
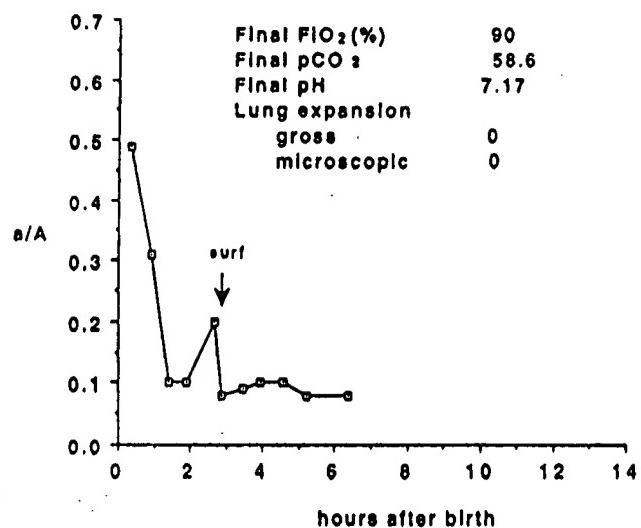


Fig. 2

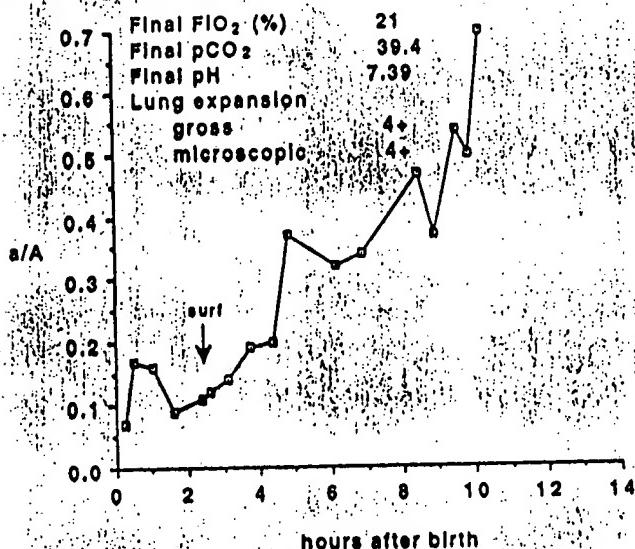
Monkey #3



Monkey #5



Monkey #6



Monkey #7

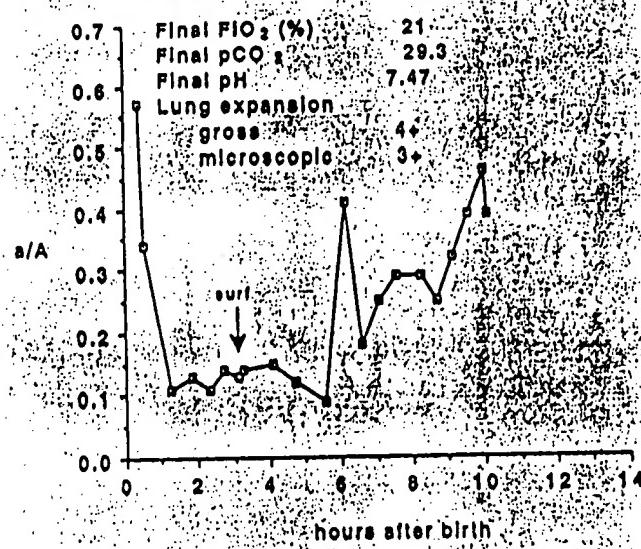
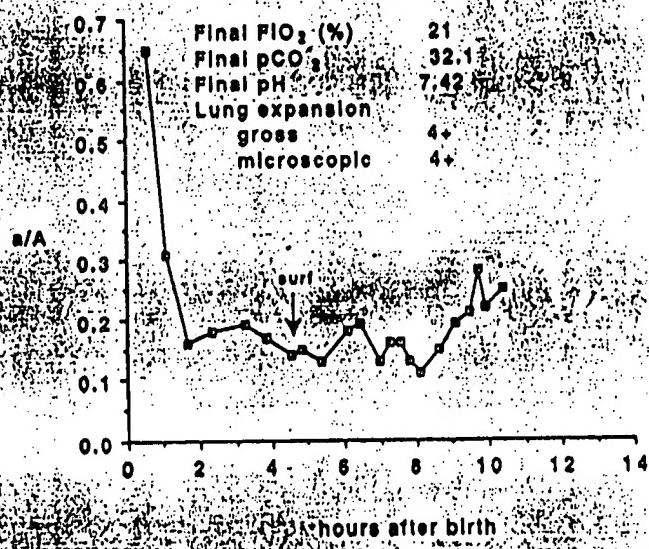
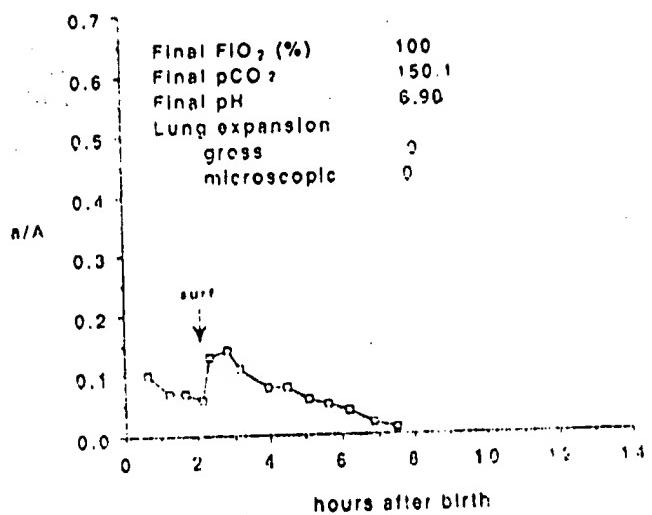


Fig. 3A

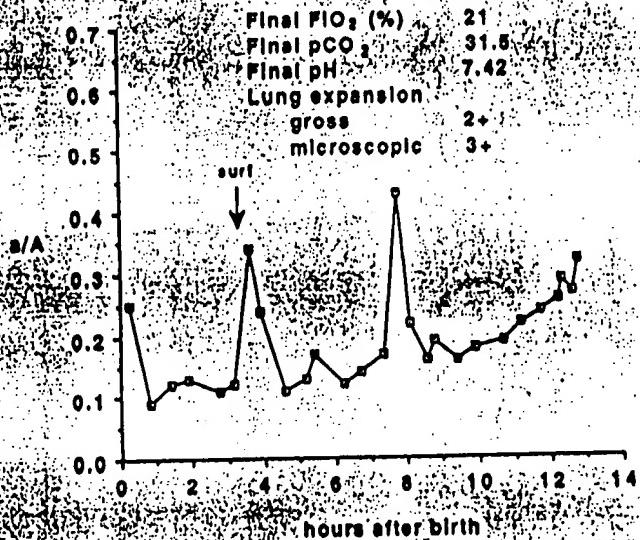
Monkey #8



Monkey #9



Monkey #10



Monkey #11

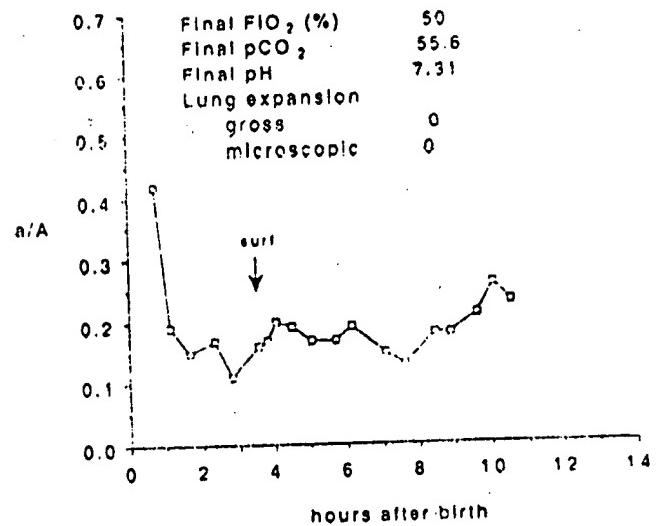
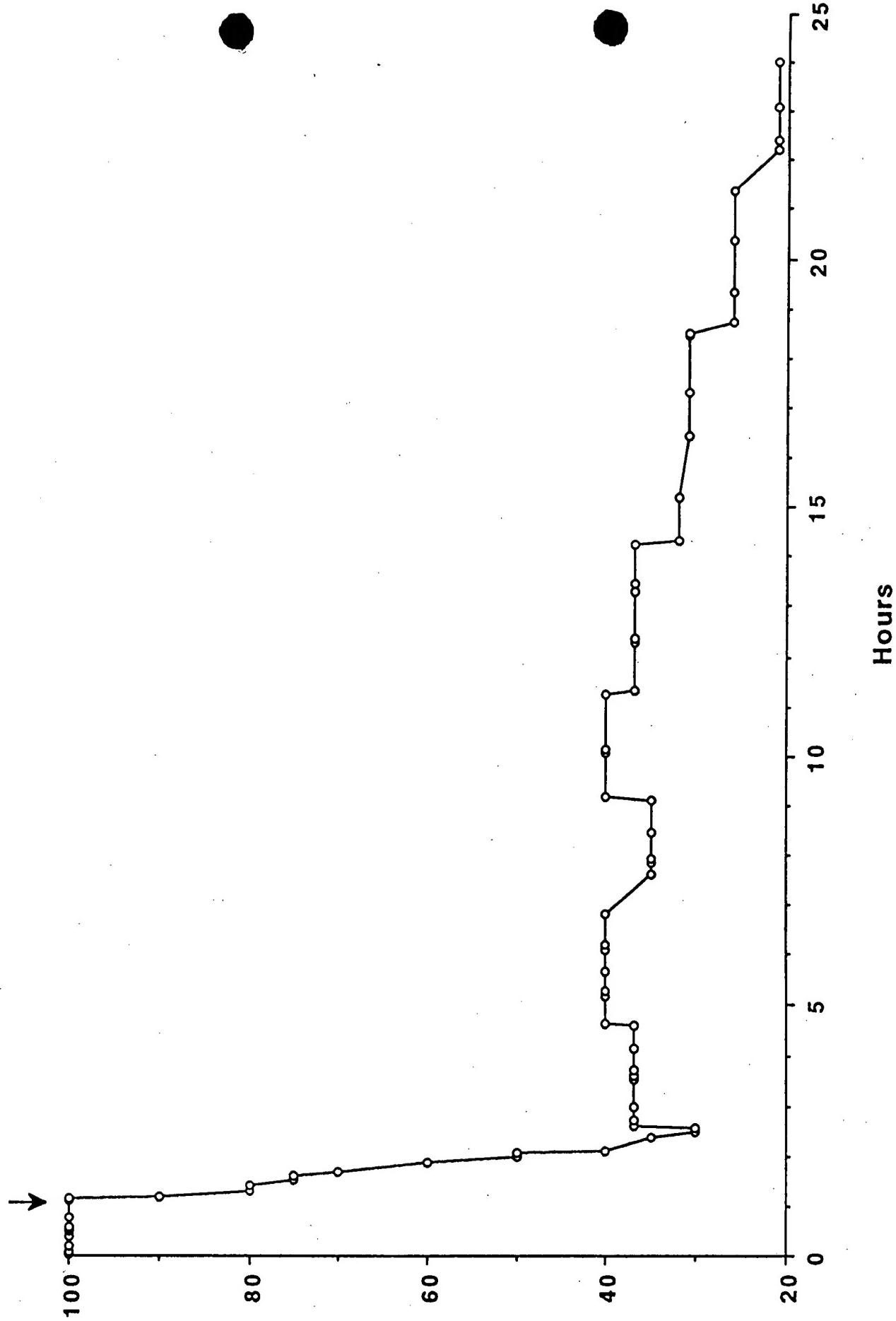


Fig. 3B

Monkey #13

peptide
surfactant



F102

Fig. 4